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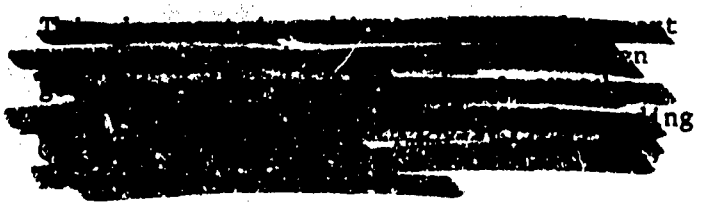

DEPARTMENT OF THE ARMY
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#253

Plague Immunology.

VI. Bacteriolysis in Plague.*

(From the State Institute of Microbiology and Epidemiology of the South-West USSR)

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Vestnik Mikrobiol., Epidemiol. i Parazitol. 16: 54-63: 1937.

The monographs of Dieudonne u. Otto and Pollitzer are the only works in which attempts have been made to combine, as far as possible, all data on plague immunology.

On the problem which is of interest to us, there is presented there a series of investigations, and the general conclusion drawn from them is that the action of antiplague serums is based on their bactericidal properties.

It would appear completely natural, therefore, to turn one's attention to a study of the mechanism of the bacteriolysins' action for the purpose of working out methods of titrating antiplague serums.

Nevertheless, until recently there have been practically no works on the investigation of bacteriolysis in plague. The reason for this circumstance is undoubtedly the fact established by Kolle, that bacteriolysins do not act in vitro. Consequently, it is necessary to carry out the titration of bacteriolysins in vivo, which makes a quantitative calculation difficult. In addition, Kolle pointed out the lack of constancy and conciseness in the fixed complement test.

The last problem was reviewed by Greval and Dalal on the basis of modern microbiological techniques. The authors come to the following conclusion: the fixed complement test may be also usable " for measuring the titer of antiserums without dependence upon the method of immunization;" it is true, the authors note, that, "this system is rated below the agglutination test."

It seemed necessary for us to reexamine the first question established by Kolle: can bacteriolysis occur in vitro in plague.

Together with this, it should be noted that there are data which indicate an absence of complement in the organism of animals infected with B. pestis. And therefore it seems important to also answer the question, does not the absence of complement in vivo constitute an impediment in a treatment with antiplague serums, and is it impossible to eliminate this insufficiency by the injection of fresh complement.

Our first task led to an attempt to produce bacteriolysis in vitro using a system differing from that of Kolle.

We assumed that if it were impossible to obtain a bacteriolytic effect (dissolving of the bacteria) properly in vitro, then it may be possible to

develop a bactericidal effect (death of the bacteria without dissolution). At first we adopted an already well known system for the resolution of this problem. In our tests it is described in the following form: we poured into each of the experimental test tubes 0.5 cm^3 of an inactivated antiplague serum, 0.5 cm^3 of an emulsion of B. pestis of a definite concentration and 0.25 cm^3 of guinea pig serum. The same ingredients went into the control of the complement, only the guinea pig serum was made inactive at 56°C for a period of 15 minutes. In addition, a general control was established consisting of inactivated complement plus inactivated normal horse serum plus a B. pestis emulsion. All the test tubes were placed into a thermostat at 28°C for 40 minutes and from each of them one droplet was taken, transferred to Petri dishes with agar and carefully rubbed in. For the first and second 24-hour periods a tabulation was made of the colonies and their character surveyed. This system proved to be unsuccessful in regards to B. pestis. The fact being that B. pestis in seedings on Petri dishes grows on common MPA only with a sufficiently huge inoculation, while not growing in small quantities. In other words, with decreasing doses of inoculation, the number of colonies does not decline in parallel with the gradual reduction of the amount of inoculated material, but falls critically as soon as it passes a known threshold. This threshold for B. pestis lies much higher, that is, it was expressed in larger quantities of the inoculation material in comparison, for example, with the intestinal group.

Consequently, we modified our system in such a manner so that for the developmentof bacteriolysis from the experimental and control test tubes (in tables 1, 2 & 3 the left side of the tables indicates these test tubes and their ingredients) after their stay in the thermostat at 28°C for a 40 minute period, the sowing was not made onto Petri dishes, but into test tubes containing 1.8 cm^3 of broth which was first poured and sterilized. As many series were taken as there were experimental and control test tubes together, and each series in turn consisted of 10 test tubes. The inoculation was made by the following method: after a thorough mixture, 0.2 cm^3 of the contents from the original test tubes was transferred into the first test tube; it was carefully mixed and again 0.2 cm^3 was transferred through a fresh pipette into another test tube and so on. Thus, a tenfold dilution was received, such as: 1: 10, 1:100 and so on to the tenth test tube, corresponding to a 1:10 milliard dilution of the original material. The culture was placed into a thermostat at 28°C and the cultures checked at the first and second 24-hour periods. (the data of this portion of the experiment are inserted on the right side of tables 1, 2 & 3 and indicate the highest dilution which still gave growth). The experiment was calculated on the basis of the fact that with a decrease of the number of microbic bodies in those test tubes containing all the ingredients necessary for bacteriolysis (immune serum + active complement) the growth will be in the lower dilutions. On the other hand, we had in mind, by means of a reduction of the high concentration of the serums, to improve the conditions for the growth of B. pestis, which could have been somewhat retarded by the high concentration of a serum, which would prevent the clarification of the true ratios. In table No. 1, test No. 1, are shown the highest dilutions which gave growth, 1:10,000 and 1:100,000. It is evident from them that there is no difference between the test tubes with the active complement (experimental) and those with the inactivated complement (control): Iersen's serum, both with the

inactivated and with the activated complement, permits the growth of B. pestis to a dilution of 1:100,000; the capsule serum permits the growth to a dilution of 1:10,000. The general control also gives a growth in a dilution of 1:10,000. It seemed possible to us, in view of the special properties of B. pestis for the accomplishment of bacteriolysis, that in addition to the bacteriolysins and the complement, there is needed some third factor, which is present in an organism, but absent in a test tube. Leucocytes could prove to be this factor. For a check of this possibility, weak of course, but one which cannot be rejected in connection with B. pestis, the following test was staged. To the test tubes, which were prepared as for the first test, were added washed-off leucocytes from a sheep and from an immune horse, 0.5 cm^3 in each test tube (table 1, tests No. 3 & 4). It is evident from the tests that whole leucocytes do not contribute to the bacteriolysis of B. pestis in vitro. In test No. 3 the growth, both in the experimental and the control testtubes, was 1:10,000; in test No. 4 the growth in all cases was 1:100,000.

Considering it possible that the capsule of B. pestis may play some role in the bacteriolysis, we arranged an experiment in the former order, but using a culture grown at 37°C and, consequently, having capsules. The hopes at this moment were also unjustified, as seen in table 1, test No. 2. Thus, the results which are condensed in table 1 definitely confirm the data of Kollo, concerning the inactivity of bacteriolysins in vitro in the presence of a sufficiently active complement.

For an explanation of the bacteriolysins' refusal to work in vitro, different experiments were demanded. The next attempt to resolve the problem emerged from such considerations as : the test with bacteriolysis in vitro was generally made with undiluted serums, but meanwhile such a system may lend itself to experiencing the so-called Neisser-Wehsberg phenomenon. It consists of the fact that in some cases bacteriolysis in vitro is possible only with diluted serums and is completely absent with the undiluted. For the purpose of taking this important indication into consideration, we staged the test of bacteriolysis in vitro in the order given below (table 2).

The primary test with controls was now made with not only the undiluted serum, but also with the diluted, that is, it consisted of 18 test tubes (6 testtubes for each control and test), bringing the dilution of the serums to 1:160. The rest of the ingredients were added to 0.5 cm^3 of the diluted serums in the same ratios, that is, 0.5 emulsion + 0.25 complement. All test tubes were placed into the thermostat. After 40 minutes a seeding was made from each test tube of the primary test into 10 test tubes, with the previous rules being observed (the careful mixing, the changing of the pipettes). Thus, each serum with the two controls gave 18 series with 10 test tubes in each, 180 in all. All of the test tubes were placed into a thermostat at 28°C and checked after 1-2 days. Four tests were staged and they gave very inconsistent results. Test No. 5 indicates that the general control gave the larger number of test tubes with growth, then came the complement control,, and the least of all was the test itself. Test No. 6 gave practically no difference between the experimental and control test tubes. Tests No. 7 and 8 even gave a reverse picture, that is, the growth claimed a larger number of dilutions in the experimental test tubes than it did in the control.

Thereby, table 2 results in showing the impossibility of drawing a positive conclusion concerning bacteriolysis in vitro with plague; although test No. 5 does indicate some influence of the bacteriolysins on B. pestis, it is not a rule, because when repeated, different results were received (tests No. 6, 7 & 8). It is possible that here is expressed the lack of constancy in the fixation of the complement in seemingly adequate conditions, as reported by Kolle.

An indication that avirulent cultures are more easily subjected to bacteriolysis than the virulent gave us occasion to stage a test with 5 strains; two avirulent (74 R and $\frac{11}{10}$ R), two virulent (286 and 217) and one heterovirulent (ZhVR). The data of this test are summarized in table 3, test No. 9. This test was staged for every strain in the same aspect as in tests No. 5, 6, 7 & 8, and since there were five strains the volume of the test was 5 times larger than each of the aforementioned tests individually.

In the left portion of the table are indicated the 18 test tubes of the primary test with their ingredients which were preliminarily placed in a thermostat at 28°C for 40 minutes in order to produce bacteriolysis, and only after this was there made a sowing from each test tube into the 10 test tubes comprising one series. This test gave negative results as there was no difference in the growth between the test tubes that were to develop the bacteriolysis and the test tubes that were to control it. The only difference in growth was between the separate strains, which was due to the intensity of the growth of the separate cultures. Thus, the ZhVR grew more intensively than the other strains and thereby produced growth in the seeding from the primary test tubes in a dilution of 1:10 milliard, whereas the other four strains acted almost identically and produced a significantly lesser intensity of growth. The last test (No. 10) in vitro was conducted in approximately the same volume and with the same five strains, plus one strain of pseudotuberculosis. To the entire system prior to its placement into the thermostat, was added 0.25 cm^3 of a plague bacteriophage. In the sowing from the primary test tubes, growth occurred in only the lower dilutions and seldom reached 1:10,000. However, there was no difference between the test tubes of the experiment and the control - the test was also negative. The fact that the strain of pseudotuberculosis gave a growth in comparatively higher dilutions than the plague strains is due to the specificity of the bacteriophage. In parallel with the tests in vitro we staged experiments on guinea pigs for the purpose of assuring ourselves of the fact that bacteriolysis actually occurs in an organism in the presence of antiplague serum and an active complement. The experiment was arranged according to the form of the Pfeiffer phenomenon. Upon injection of the antiplague serum into the guinea pigs, with a subsequent intra peritoneal infection, we were able to state within one hour after the infection, through the use of suspended droplets, the typical form of bacteriolysis in the exudate. This appearance was less marked in the control animals. Thus, bacteriolysis in vivo is completely possible, in contrast to the experiment staged in vitro. Until now this fact had not received the foregoing demonstration. Thus, Kolle was forced to acknowledge that though injecting immune serum resulted in the bacteria being destroyed, still the mechanism of the destruction is not in accordance with ordinary bacteriolysis and must be considered as an action of a yet unknown "antiinfection" factor.

In table 4 are presented all data received by us as a result of treatment with antiplague serums with the addition of active complement. From tests No 1, 2 (part 9) and 5 it is seen that in the guinea pigs treated with antiplague serum and complement, the average percentage survival is larger than in the animals treated with only serum alone. From test No. 2 we see that the percentage survival in them also is larger than in those treated with only antiplague serum. Test No. 3 produces a completely different picture, because in it the percentage survival in the guinea pigs treated with the antiplague serum and complement is less than in the guinea pigs treated with only the anti-plague serum. And in test No. 6 the percentage survival in both cases is one and the same.

Conclusions

1. Regardless of the many varied attempts to produce bacteriolysis in vitro with plague, we did not succeed in doing so, and only in one test did we observe any reaction of the bacteriolysins on B. pestis, however this reaction was inconsistent (negative results of the other tests). It is possible that here is expressed the lack of constancy in the fixation of the complement under seemingly adequate conditions to which Kolle made reference.

2. The experiments in vivo confirmed the data that in vivo bacteriolysis in plague actually takes place.

3. The injection of complement together with antiplague serum into an animal organism gave us inconstant data, which changes in accordance with the method used in infecting the animal and the virulence of the material, but in any case it was possible to note an influence of the complement upon the course of the infection.

4. We do not consider the negative data received in the staging of the experiments in vitro as final, and think that the failure lies in an imperfection of the present system being used by us and in a series of as yet unexplored factors which can contribute to the bacteriolysis in vitro in plague.

* Footnote

Report I. Zhukov-Verezhnikov, N. and Lipatova, T. - Vestnik Microb., Epidemiol. and Parazit., XII, No 4, 1933.

Report II. Khvorostukhina, M., same publication, XIII, No 1, 1934.

Report III. Lipatova, T., same publication, XIII, No 3, 1934.

Report IV. Zhukov-Verezhnikov, N, Faddeeva, T. and Lipatova, T., same publication, XIV, No 2, 1935.

Report V. Kitin, S., same publication, XV, No 2, 1936.

Literature

Dieudonne u. Otto: Kolle, Kraus, Uhlenhuth "Handbuch der pathogenen Mikroorganismen" p. 255-303.

Pollitzer: W u Lien-Teh, Chun, Pollitzer and Wu: "Plague. A manual for medical and Public Health Workers."

Grevala. Dalal: Ind. J. of Med. Res.

Table 1

The development of bacteriolysis in vitro through seeding the primary material into test tubes with broth.

No of test tube	Special features of the separate tests. → / Ingredients of the test and control test tubes.	Test No. 1 — Culture grown at 28°C	Test No. 2 — Culture grown at 37°C	Test No. 3 — Culture grown at 28°C plus Sheep leukocytes.	Test No. 4 — Culture grown at 28°C plus immune horse leukocytes.	Note
1	Test Iersen serum. Emulsion. Complement.	10^5	10^6	10^4	10^5	Figures indicate that highest dilution which still produced a growth characteristic for B. pestis.
2	Control Iersen serum. Emulsion. Inactivated complement.	10^5	10^7	10^4	10^5	
3	Test Capsule serum. Emulsion	10^4	10^5	10^4	not made	
4	Control Capsule serum. Emulsion. Inactivated complement.	10^4	10^5	10^4	not made	
5	General Control Normal serum. Emulsion. Inactivated serum.	10^4	10^5	10^4	10^5	

Table 2

The development of bacteriolysis in vitro by means of sowing the primary material (including diluted serum in addition to the whole serum) into test tubes with broth.

No. of test tubes	Designation	Ingredients		Test No 5	Test No 6	Test No 7	Test No 8	Note
		Individual	General					
1	Test	Whole serum	Emulsion. Complement	10^1	10^8	10^9	10^3	Figures indicate the höghest dilution of the primary material which still gave growth to B.pestis.
2		1:10	"	10^3	10^8	10^6	10^2	
3		1:20	"	10^3	10^7	10^9	10^3	
4		1:40	"	10^3	10^6	10^7	10^4	
5		1:80	"	10^3	10^7	10^7	10^4	
6		1:160	"	10^1	10^7	10^7	10^4	
7	Control I	Whole serum	Emulsion Inact. complement.	10^2	10^7	10^6	10^3	
8		1:10	"	10^2	10^7	10^5	10^2	
9		1:20	"	10^2	10^7	10^5	10^2	
10		1:40	"	10^4	10^7	10^4	10^2	
11		1:80	"	10^7	10^7	10^5	10^2	
12		1:160	"	10^7	10^5	10^5	10^1	
13	Control II	Normal whole serum	"	10^6	10^6	10^3	10^2	
14		1:10	"	10^8	10^8	10^4	10^2	
15		1:20	"	10^8	10^8	10^2	10^2	
16		1:40	"	10^7	10^7	10^2	10^2	
17		1:80	"	10^7	10^7	10^2	10^2	
18		1:160	"	10^7	10^7	10^2	10^2	*

Table 3

Development of bacteriolysis in vitro with virulent, avirulent and heterovirulent strains.

No. of test tubes	Designation	Different strains in the test: Ingredients — Individual	General	Test No. 9: 286	217	74R	11_R / 10	ZhVR	Test No. 10 with an addition of 0.25 cm^3 of a bacteriophage: 286	217	74R	11_R / 10	ZhVR	SR
1	Test	Whole serum	Emulsion. Complement.	10^6	10^3	10^5	10^4	10^9	10^2	10^3	10^1	10^2	10^1	10^4
2		1:10	"	10^4	10^4	10^6	10^5	10^8	10^2	10^2	10^2	10^2	10^2	10^3
3		1:20	"	10^6	10^3	10^7	10^8	10^9	10^2	10^2	10^2	10^3	10^2	10^2
4		1:40	"	10^5	10^4	10^6	10^4	10^9	10^2	10^2	10^1	10^3	10^2	10^4
5		1:80	"	10^7	10^4	10^6	10^5	10^7	10^1	10^2	10^2	10^2	10^2	10^4
6		1:160	"	10^6	10^6	10^5	10^5	10^{10}	10^1	10^2	10^2	10^4	10^3	10^3
7	Control I	Whole serum	Emulsion. Inact. complement.	10^7	10^6	10^6	10^5	10^{10}	10^3	10^2	10^2	10^2	-	10^3
8		L:10	"	10^6	10^5	10^5	10^4	10^9	10^1	10^2	10^2	10^2	-	10^3
9		1:20	"	10^7	10^5	10^6	10^4	10^9	10^2	10^2	10^2	10^2	-	10^3
10		1:40	"	10^8	10^6	10^6	10^5	10^{10}	10^2	10^4	10^2	10^3	10^1	10^3
11		1:80	"	10^8	10^5	10^7	10^5	10^{10}	10^2	10^5	10^4	10^3	10^1	10^3
12		1:160	"	10^8	10^5	10^6	10^4	10^{10}	10^2	10^2	10^2	10^4	10^2	10^3
13	Control II	Normal whole serum.	"	10^6	10^5	10^6	10^5	10^{10}	10^4	10^2	10^4	10^3	10^2	10^4
14		1:10	"	10^6	10^6	10^6	10^5	10^{10}	10^2	10^3	10^4	10^2	10^3	10^4
15		1:20	"	10^4	10^4	10^6	10^7	10^{10}	10^2	10^3	10^2	10^2	10^1	10^4
16		1:40	"	10^8	10^4	10^7	10^7	10^9	...	10^3	10^3	10^2	-	10^4
17		1:80	"	10^7	10^4	10^4	10^8	10^{10}	-	10^2	10^4	-	-	10^4
18		1:160	"	10^5	10^4	10^5	10^8	10^9	10^1	10^2	10^4	-	10^3	10^4

Table 4

The influence of complement on the length of life and percentage survival of guinea pigs. The complement is injected in a quantity of 3 cm^3 within two hours after the simultaneous injection with a culture of 1:10 of an infected loop and serum (5 cm^3).

Nos. of the tests	No. of animals	Method of infection	Virulence of the culture.	Length of life in days (injected materials)			Percentage of Survival. (injected materials)	
				Serum & Complement	Serum	Control	Serum & Complement	Serum
Test No. 1	9	Subcutan.	Strong	10	6	4	=	=
Part 1 Test No. 2	6	Subcutan.	Weak	-	11	10	33 %	16%
Part 2	9	Intraper.	Weak	12.5	12	9	11 %	0
Test No. 3	15	Intraper.	Med.	8	9.5	6	*	=
Test No. 4	18	Subcutan.	Med.	9	14.5	6	5.2 %	11 %
Part 1 Test No. 5	6	Subcutan.	Weak	14	10.5	11	-	-
Part 2 ***	6	Subcutan.	Strong	10.5	9	5	-	-
Test No. 6	6	Subcutan.	Med.	-	=	7	33 %	33 %

*** Complement was injected after 4 hours.